

THE PATHOGENESIS OF CHRONIC INFLAMMATION IN EXPERIMENTAL ANTIGEN-INDUCED ARTHRITIS

II. PREFERENTIAL LOCALIZATION OF ANTIGEN-ANTIBODY COMPLEXES TO COLLAGENOUS TISSUES*

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The antigen-induced synovitis of Dumonde and Glynn (1) was initially described as a chronic inflammatory response of rabbits previously immunized to homologous and heterologous fibrin. Subsequently, the induction of a similar chronic synovitis after intra-articular (IA)¹ injection of soluble proteins such as egg albumin (EA) and bovine serum albumin (BSA) in immune rabbits was described (2). In a previous paper,² we have reported that levels of immunoglobulin (Ig) synthesis by synovium with this type of synovitis induced by EA were well maintained for at least 6 wk after induction. The immunoglobulin synthesized consisted largely of specific antibody to the injected antigen. These findings indicated that the sustained local immune response may be maintained by persisting antigen retained in the joint and released at a very slow rate.

The present paper describes immunofluorescence and antigen elution studies which have localized the antigen in the joint and presents a mechanism for its persistence. The findings reported suggest that intra-articular antigen plays a highly significant role in the synovial inflammatory response in the following respects: (a) It serves as the direct stimulus for the local synthesis of antibody in the synovial tissue and (b) it subsequently engages in the formation of com-

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¹ *Abbreviations used in this paper:* BSA, bovine serum albumin; CFA, complete Freund's adjuvant; EA, egg albumin; IA, intra-articular; IF, immunofluorescence; Ig, immunoglobulin; PBS, phosphate-buffered saline solution.

² Cooke, T. D., and H. E. Jasin. 1972. The pathogenesis of chronic inflammation in experimental antigen-induced arthritis. I. The role of antigen on the local immune response. Unpublished manuscript.

plement-binding antigen-antibody complexes which may play a major part in the production and maintenance of the synovial inflammation.

Materials and Methods

Materials.—Rabbits, EA and BSA, rabbit anti-BSA, goat anti-rabbit Ig, complete Freund's adjuvant (CFA), and ^{125}I were obtained and prepared as described previously.² Microcrystalline monosodium urate crystals were obtained from Dr. Jay Seegmiller of the National Institutes of Health. Fluorescein-conjugated antisera were obtained from Hyland Laboratories, Los Angeles, Calif. Guinea pig anti-rabbit C3 was prepared by the method of Mardiney and Müller-Eberhard (3).

Induction of Arthritis.—Procedures identical to those described in a preceding paper² were followed. In these experiments, BSA- ^{125}I or EA- ^{125}I , labeled as described previously,² were used for the IA injections in doses ranging from 15 to 30×10^6 cpm.

Crystal-induced acute synovitis was produced by IA injection of 5 mg of a sterile suspension of monosodium urate crystals 6–30 hr before resection of the joint tissues.

Measurements of EA- ^{125}I or BSA- ^{125}I Tissue Radioactivity.—The animals were exsanguinated under pentobarbital anesthesia and then killed by air embolism. Both knee joints were completely removed. By careful dissection, the following tissues were separated: articular cartilage shavings, menisci, intra-articular ligaments, synovium and fat pad, juxta-articular bone with remnants of articular cartilage and ligamentous tissue which could not be dissected away. 2-ml volumes of chopped tissue were counted in a Gammaguard 150 Spectromatic well-scintillation counter (Tracerlab, Richmond, Calif.).

Radioautography.—Specimens of synovium, intra-articular ligaments, menisci, and articular cartilage, obtained at intervals up to 6 wk after IA challenge with EA- ^{125}I or BSA- ^{125}I , were fixed in 10% buffered formalin immediately after removal from the joint. Deparaffinized histologic sections were dipped in NTB3 photographic emulsion (Eastman Kodak, Rochester, N.Y.) and exposed for periods of 1–3 wk. They were then developed and stained with hematoxylin and eosin (H and E). The optimal time for exposure was between 2 and 3 wk.

Elution of ^{125}I -Labeled Antigens from Collagenous Tissues.—The freshly excised collagenous tissue (hyaline cartilage, menisci, and ligaments) from arthritic knees injected with radioactive antigen 2, 4, and 6 wk previously were weighed, minced, and extracted with 10 volumes of phosphate-buffered saline, pH 7.2 (PBS), with continuous stirring at 4°C for 15 hr. Subsequently, the tissues were extracted consecutively with similar volumes of 0.2 M glycine hydrochloride, pH 3.2 containing 2 M NaCl; 3 M Mg Cl₂ in order to remove protein-polysaccharides (4); and finally with 5 M guanidine in 0.1 M tris (hydroxymethyl) aminomethane (Tris) buffer, pH 7.2, containing BSA in a concentration of 1 mg/ml. The bulk of the eluted radioactivity was found in the 5 M guanidine extracts. These were dialyzed against PBS and coprecipitated with BSA-anti-BSA or EA-anti-EA at equivalence. The precipitates were washed as described previously² and their radioactivity determined.

Immunofluorescence Studies on Excised Joint Tissues.—Fresh joint tissues from arthritic rabbits induced 2, 4, and 6 wk previously by EA or BSA were washed for at least 30 min in Eagle's minimal essential medium or PBS. They were immersed in Tissue-Tek (Ames Co., Elkhart, Ind.), placed in small plastic containers, and then snap-frozen in liquid nitrogen and stored at -70°C until sectioned. Sections were cut at 2–6 μm and applied to gelatin-coated slides. Representative sections were stained with H and E stain. Sections for immunofluorescence (IF) were kept moist in sealed boxes and then washed in PBS for 60–90 min. Conjugated antiserum, diluted sufficiently to reduce nonspecific staining, was applied for 20–30 min. Guinea pig anti-serum to rabbit C3 was similarly applied, sections washed in three 10-min changes of PBS, and then stained with diluted fluorescein-conjugated goat anti-guinea pig Ig. After washing, sections were mounted with 90% glycerol in PBS and read within 24 hr using a Leitz Ortholux microscope (E. Leitz, Rockleigh, N.J.) and Osram HBO-200 ultra-

violet light source (Macbeth Corp., Newburgh, N.Y.). A UG-I exciting filter and 430K and 460K barrier filters were used. Photomicrographs were taken with a Leitz Orthomat camera on high-speed Ektachrome film (Eastman Kodak). In order to test the specificity of the IF staining, blocking procedures were carried out in each instance with unconjugated antiserum applied for 1 hr, after which conjugated antiserum was added and the sections further incubated for 20 min. In addition, the fluorescein-conjugated reagents were also absorbed with the specific antigen before use in order to establish the specificity of the IF staining.

RESULTS

Localization of ^{125}I -Labeled Antigen to IA Collagenous Tissue—The joint tissues from animals with arthritis of 2 and 4 wk duration were dissected and their radioactivity measured. The findings in an experiment using EA- ^{125}I as

TABLE I
*Distribution of EA- ^{125}I * in Right Knees of Four Immune Rabbits with Arthritis Induced by Bilateral IA Injection of 2.5 mg of EA, EA- ^{125}I Injected into Right Knees Only*

Duration of arthritis	cpm		Distribution of radioactivity in the rt. knee			
	Right knee	Left knee	Articular cartilage	Menisci and ligaments†	Juxta-articular bone‡	Synovium
	Per cent of total					
<i>wk</i>			%	%	%	%
2	27,320	12	19	38	39	4
	21,530	134	11	22	55	12
4	11,410	8	14	41	35	2
	31,100	11	8	36	54	2

* 30×10^6 cpm were injected.

† Intra-articular ligaments: cruciates, collaterals, and quadriceps fibrocartilaginous plate.

‡ Juxta-articular bone with residual articular cartilage and ligaments that had not been dissected away.

|| Includes fat pad.

the label are seen in Table I. Approximately half of the radioactivity is seen to be localized to avascular, highly collagenous tissue, within the joint, i.e., articular cartilage, menisci, and ligaments. In marked contrast, the synovium and fat pad retained a mean of 5% of the total counts. The remaining radioactivity was found associated with the juxta-articular bone, to which some articular cartilage and the origins of the intra-articular ligaments remained attached. The findings in a subsequent study using BSA- ^{125}I , injected into the knees of rabbits immune to BSA are seen in Table II. A similar distribution of radioactivity was found, although there appeared to be some preferential localization of BSA- ^{125}I in ligamentous tissue. The collagenous tissue, i.e. cartilage, menisci, and ligaments, accounted for more than 70% of the radioactivity retained. In addition, a mean of 18% of the radioactivity was localized in juxta-articular bone which contained remnants of ligaments and cartilage. These levels did not change significantly during the time of observation;

the general pattern of distribution of radioactivity shown in the collagenous structures was maintained even though the duration of synovitis at the time of sacrifice and the dose of antigen injected had varied. In the synovium and fat pad, however, the mean value of 8% of the antigen found soon after injection fell to less than 1% by 6 wk.

TABLE II
Distribution of Radioactivity in Knees of Immune Arthritic Rabbits Injected in the Right Knee with 5 mg of BSA-¹²⁵I and in the Left Knee with 2.5 mg of BSA-¹²⁵I

No. of rabbits	Duration of arthritis	Side	Distribution of radioactivity				
			Articular cartilage	Menisci	IA ligaments*	Juxta-articular bone†	Synovium‡
			Per cent of total				
	wk		%	%	%	%	%
2	2	Right	3	30	43	19	5
		Left	15	24	34	20	7
		Right	17	32	23	18	10
		Left	21	31	29	10	9
1	4	Right	3	21	55	19	2
		Left	6	22	49	21	2
1	6	Right	—	23	44	33	0.1
		Left	6	20	55	18	0.5

* Intra-articular ligaments: cruciates, collaterals, and quadriceps fibrocartilaginous plate.

† Juxta-articular bone with residual articular cartilage and intra-articular ligaments.

‡ Includes fat pad.

|| Includes the articular cartilage.

TABLE III
Elution of Radioactivity from Collagenous Joint Tissues of Rabbits Induced by BSA-¹²⁵I

Duration of arthritis	Per cent of total radioactivity eluted*			
	PBS	Glycine-HCl pH 3.2	3 M MgCl ₂	5 M guanidine
wk				
2	0.3	0.4	4.0	66
4	0.6	N.D.‡	1.5	41
6	0.3	0.1	3.1	40

* Hyaline cartilage, menisci, and ligaments.

‡ N.D., not done.

Elution and Characterization of Radioactive Antigen.—The collagenous joint tissues, consisting of articular cartilage shavings, menisci, and IA ligaments were sequentially extracted with PBS, glycine-HCl, pH 3.2, 3 M MgCl₂, and 5 M guanidine. The data in Table III show that the radioactive material from BSA-¹²⁵I-induced joints was firmly bound since extraction with PBS, acid buffer, and 3 M MgCl₂ resulted in the elution of negligible amounts of radioactivity. Treatment with 5 M guanidine solution, on the other hand, resulted

in solubilization of 40–66% of the starting radioactive material. Aliquots of the 5 M guanidine eluate were dialyzed against PBS and coprecipitated with anti-BSA or anti-EA at immune equivalence. It can be seen in Table IV that practically all the eluted radioactivity was precipitated with the specific antigen-antibody system, indicating that it had remained antigenically intact for at least 6 wk. Tissues from arthritic joints induced by EA were also extracted sequentially and the results obtained were similar, i.e., 5 M guanidine eluted the bulk of radioactivity. However, this treatment produced extensive, irreversible denaturation of native carrier EA, and it was not possible to use immune precipitation of the eluates to characterize this antigen.

Localization of Radioactive Antigen by Radioautography.—Deparaffinized histologic sections of tissues from the knee joints of animals injected with ^{125}I -labeled antigens were exposed to the photographic emulsion and then developed. The silver grains were found thickly clustered on the surface of menisci, articular cartilage, and within intra-articular ligaments (Figs. 1–3).

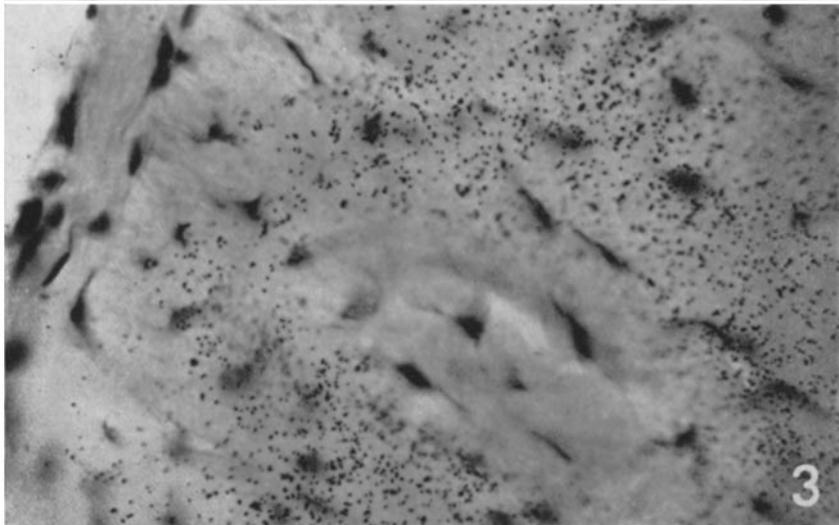
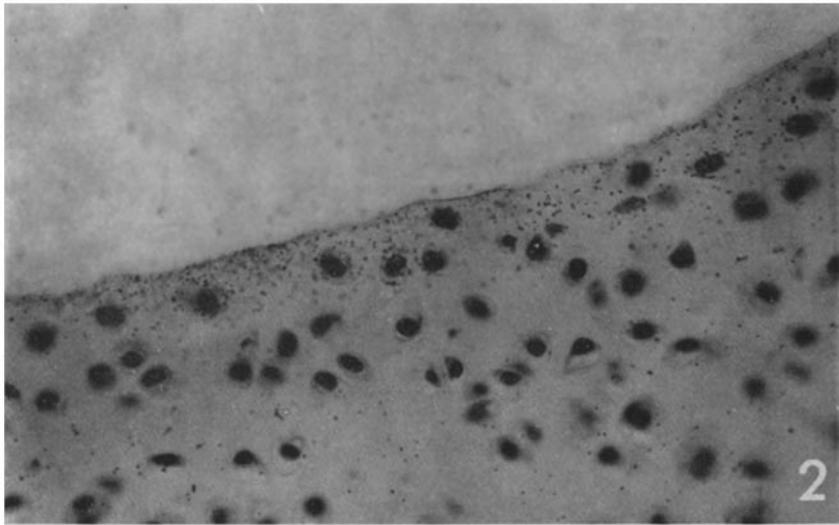
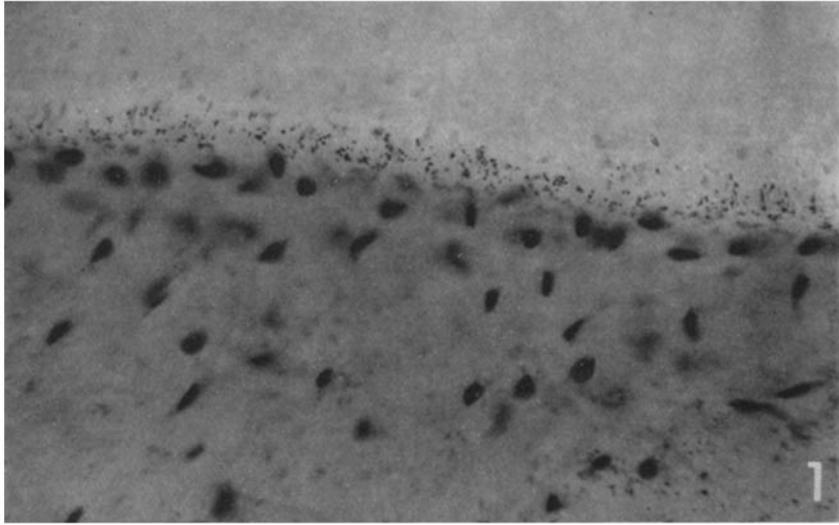
TABLE IV
*Immune Precipitation of Radioactive Material Eluted from
BSA- ^{125}I -Induced Joints by 5 M Guanidine*

Duration of arthritis	Per cent of total radioactivity precipitated with	
	BSA-anti-BSA	EA-anti-EA
<i>wk</i>		
2	90	3.8
4	82	0
6	92	0

The sections of synovium from the same joints showed no significant localization of grains above background. In one section, grains were seen overlying macrophages in synovium but this was a very occasional finding.

Localization of Antigen by Immunofluorescence.—This was examined in cartilage and menisci using fluoresceinated rabbit anti-BSA (Fig. 4). Specific fluorescence was observed to be localized to the superficial layers of hyaline cartilage and menisci in a pattern that was similar to the distribution of radioactivity seen by radioautography.

In Vitro Uptake of Antigens- ^{125}I by Menisci from Arthritic Joints.—The localization of radioactive antigen to the surface of collagenous tissue in the joint was an unexpected finding. It suggested that the deposited antigen might be complexed with specific antibody. That this was indeed the case was demonstrated by the in vitro selective binding of antigens- ^{125}I to collagenous tissue from arthritic joints. A representative experiment is shown in Table V. Whole menisci from a normal rabbit and from a rabbit with immune synovitis induced 8 wk before with BSA were extracted for 16 hr at 4°C with PBS containing 5% normal rabbit serum and then incubated with 1-ml aliquots of



EA- ^{125}I or BSA- ^{125}I , containing 5% normal rabbit serum. After 1 hr of incubation, they were washed with large volumes of PBS-serum. Only menisci from the arthritic animal bound significant amounts of the homologous antigen at two different concentrations.

Identification of Immunoglobulin and C3 on the Articular Surface of Joint Collagenous Tissue.—Frozen sections of articular cartilage, menisci, ligaments, and synovium were prepared from joints resected at intervals up to 6 wk after intra-articular induction of arthritis with EA or BSA. They were stained using direct and indirect immunofluorescence techniques. All the collagenous

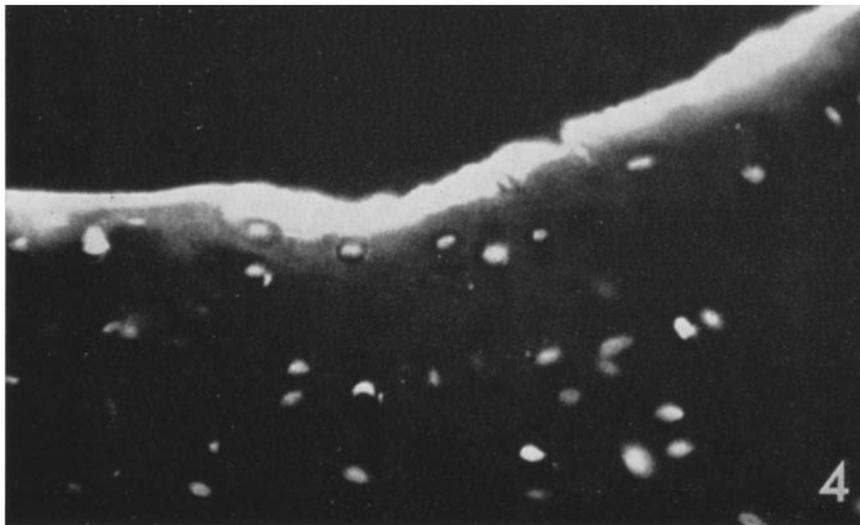


FIG. 4. Photomicrograph of articular cartilage from an arthritic joint induced 4 wk previously by 2.5 mg of BSA. Staining with fluorescein-conjugated rabbit anti-BSA, diluted 1:6. Positive staining in the surface layers is seen. Compare with radioautograph, Fig. 2. $\times 250$.

tissues from antigen-induced joints with 2–6-wk old arthritis showed positive staining for rabbit Ig on their articular surface (Figs. 5–8). The immunofluorescent staining had a similar distribution to that of the silver grains in the

FIG. 1. Radioautograph of meniscus from a rabbit with synovitis induced 4 wk previously with 2.5 mg of EA- ^{125}I . Note the relatively normal appearance of cartilage and thick clustering of silver grains in the surface layers. $\times 250$.

FIG. 2. Radioautograph of articular cartilage from a rabbit with synovitis induced 4 wk previously by IA injection of 2.5 mg of BSA- ^{125}I . Note the surface localization of silver grains in a patchy distribution. $\times 250$.

FIG. 3. Radioautograph of IA ligament, stained with H and E, from a rabbit with synovitis induced 2 wk previously by IA injection of 2.5 mg of BSA- ^{125}I . Radioactivity is found dispersed throughout the ligament. $\times 250$.

radioautographs of these tissues (Figs. 1-3). C3 was identified in the joints induced by BSA in the superficial layers of the articular surface in the same position as Ig (Fig. 9) and, as previously shown, BSA.

Menisci and articular cartilage from rabbits with an acute synovitis induced by IA injection of 5 mg of urate crystals and tissue from joints injected with saline solution were used as controls. These tissues showed no specific staining (Figs. 10-12).

DISCUSSION

In these experiments, a chronic experimental antigen-induced arthritis has been investigated to explore the mechanisms by which chronic inflammation is maintained after IA injection of antigen in previously immunized rabbits. In a previous paper,² it was shown (a) that specific antibody to the injected

TABLE V
In Vitro Binding of BSA-¹²⁵I by Menisci from Normal Rabbits and Rabbits with Arthritis Induced by BSA 8 Wk Previously

Antigens incubated	Antigen concentration	Source of menisci	Tissue weight	Uptake by menisci	Corrected uptake*
	<i>μg/ml</i>		<i>mg</i>	<i>cpm/mg</i>	<i>ng/mg</i>
BSA- ¹²⁵ I†	5	BSA arthritis	55	13.5	1.45
	5	Normal	55	2.0	
EA- ¹²⁵ I‡	5	BSA arthritis	66	0.15	0
	5	Normal	50	0.22	
BSA- ¹²⁵ I§	100	BSA arthritis	18	3.6	6.1
	100	Normal	18	1.2	

* Corrected for nonspecific uptake by normal meniscus.

† Specific radioactivity: BSA-¹²⁵I: 7800 cpm/μg; EA-¹²⁵I: 770 cpm/μg.

§ Specific radioactivity: BSA-¹²⁵I: 390 cpm/μg.

antigen was synthesized in the synovial tissue for prolonged periods of time and (b) that the injected antigen was selectively retained in the joint and released very slowly.

In the present work, it has been attempted to identify the retained antigen in the joint and to establish its sites of deposition. Unexpectedly, most of the retained antigen-¹²⁵I was found to be consistently present in highly collagenous tissues such as menisci, IA ligaments, and articular cartilage. Radioautographic examination of tissue sections indicated that a major proportion of the antigen was present in a patchy distribution in the superficial layers of the articular surfaces of these tissues. This finding was confirmed using fluoresceinated specific antisera which demonstrated the localization of antigen in a very similar pattern. In marked contrast was the very small fraction of the total articular radioactivity found in synovium. Furthermore, the synovial fraction decreased markedly over a 6 wk period to less than 1% of the total whereas that in collagenous tissue remained constant, amounting to at least 70% of the

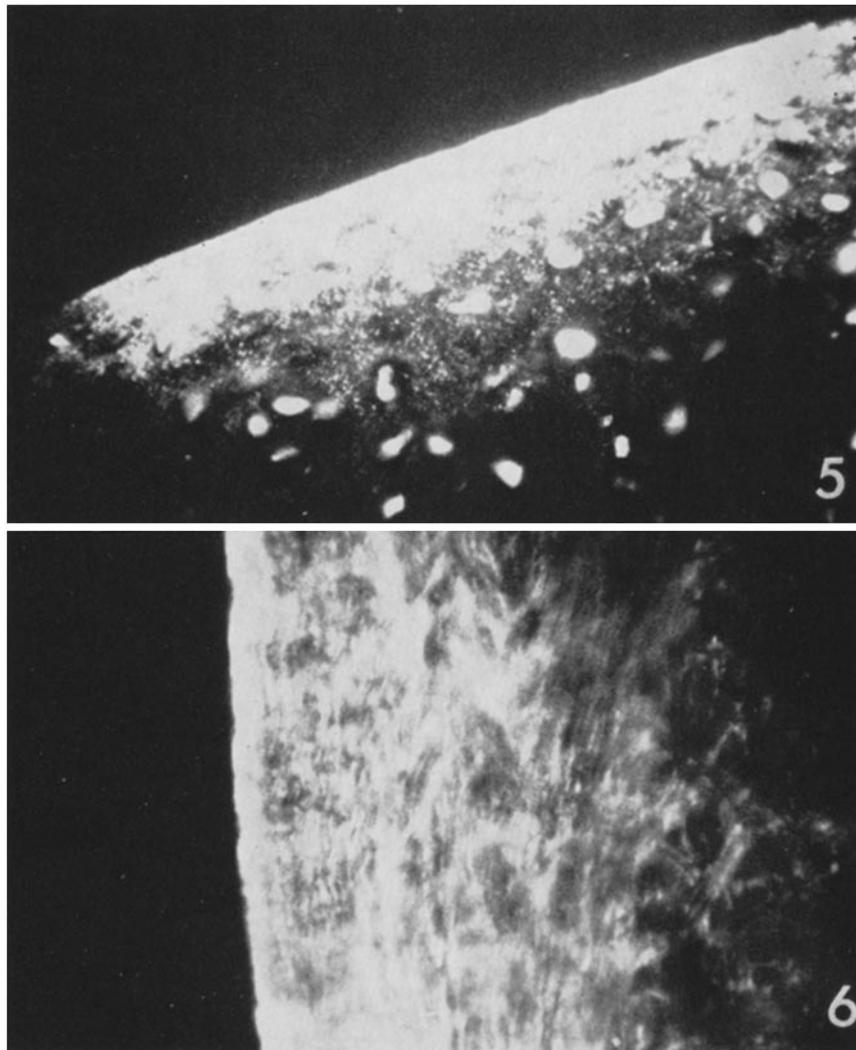


FIG. 5. Meniscus of rabbit with immune synovitis induced 6 wk previously by 2.5 mg of EA, stained with fluorescein-conjugated goat anti-rabbit Ig, diluted 1:4. Note the intense staining on the articular surface. $\times 250$.

FIG. 6. Meniscus of rabbit with immune synovitis induced 2 wk previously by 2.5 mg of BSA. Stained with fluorescein-conjugated goat anti-rabbit Ig, diluted 1:5. Wavy band-like pattern of the staining suggests localization to collagen near the articular surface. $\times 225$.

total joint radioactivity. Since radioautographs of the synovium failed to reveal significant radioactivity, the possibility of selective concentration of antigen in a small portion of this tissue, such as the synovial lining layer, was ruled out.

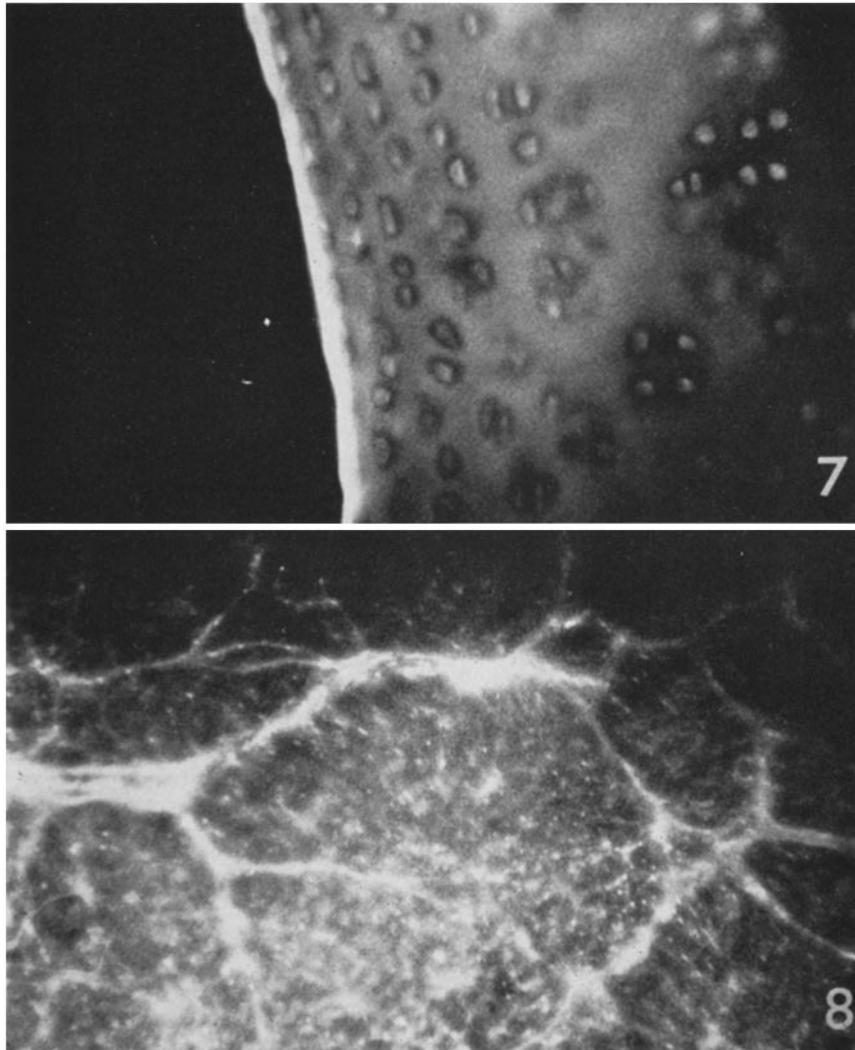


FIG. 7. Articular cartilage shaving of rabbit with immune synovitis induced 4 wk previously by 2.5 mg of BSA, stained with fluorescein-conjugated goat anti-rabbit Ig, diluted 1:5. Note the marked surface staining of cartilage. $\times 250$.

FIG. 8. Intra-articular ligament of rabbit with immune synovitis of 2 wk duration induced by BSA, stained with fluorescein-conjugated anti-rabbit Ig diluted 1:5. Note the surface staining and diffuse interfascicular staining. $\times 400$.

The localization of antigen in avascular, hypocellular collagenous tissue provides a reasonable explanation for the very slow elimination rates observed between 1 and 4 wk after injection. The relatively rapid disappearance from the synovium, on the other hand, is presumably a consequence of phagocytosis

and digestion of antigen, either free or in antigen-antibody complex with ready escape of the digestion products into blood and lymph. Indirect support for this concept is provided by the finding by Currey et al. (5) that synovectomy performed on rabbits with a severe antigen-induced arthritis was unable to modify the subsequent course of the chronic inflammatory process which reappeared concurrently with synovial regeneration. Since synovectomy usually avoids removal of ligamentous and cartilaginous elements, the major fraction of the retained antigen had presumably not been removed.

That the radioactivity persisting in the present experiments did represent

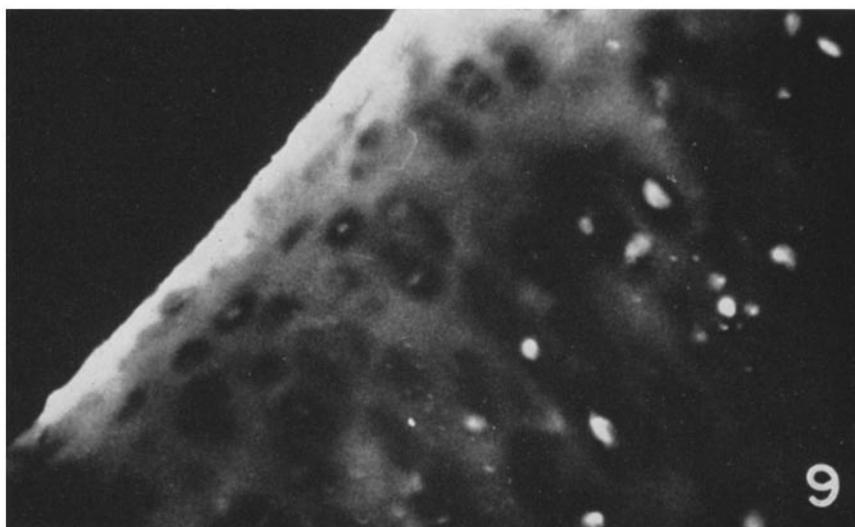
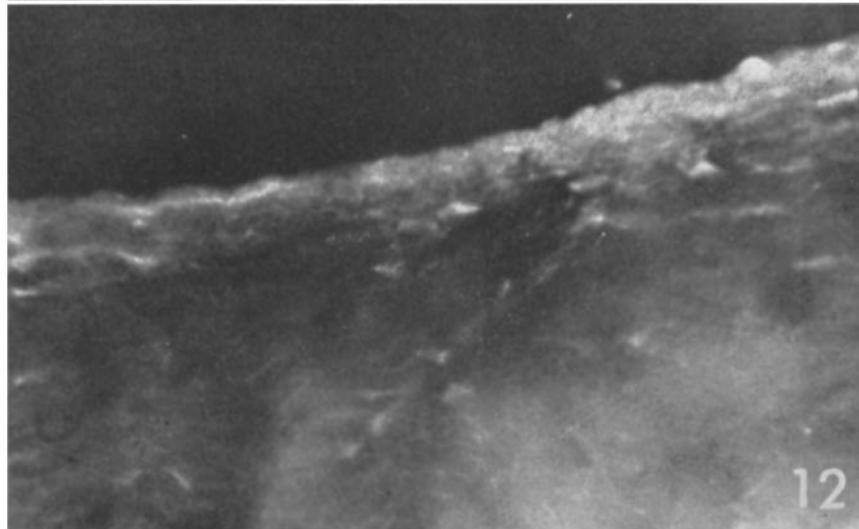
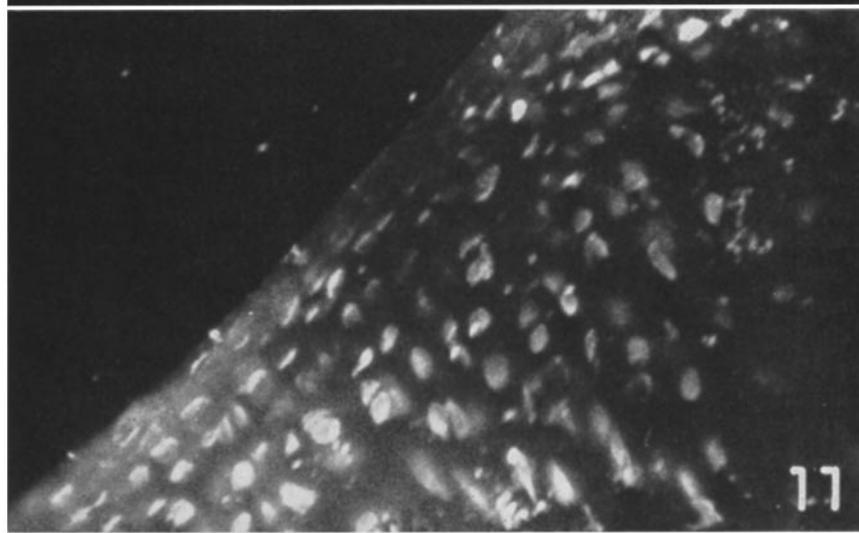
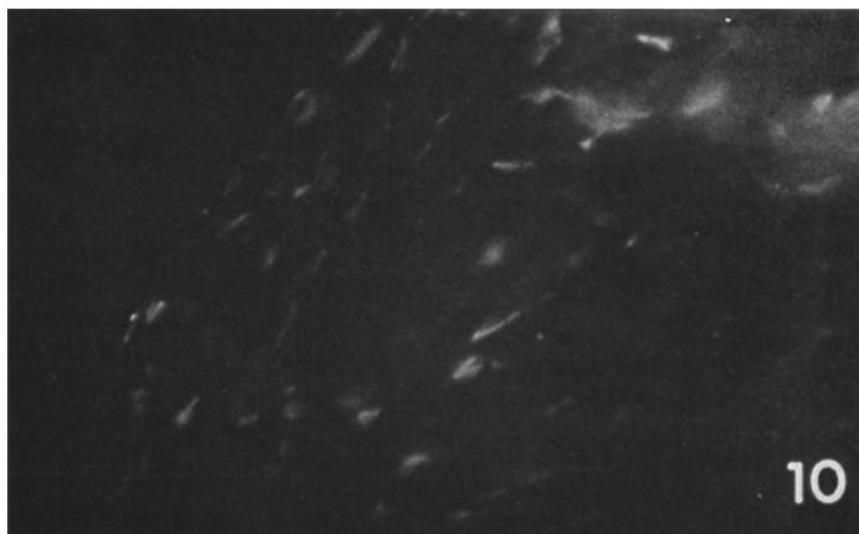


FIG. 9. Photomicrograph of meniscus from arthritic joint induced 6 wk before by 2.5 mg of BSA. Stained with guinea pig anti-rabbit C3, diluted 1:2, and then by fluorescein-conjugated rabbit anti-guinea pig Ig diluted 1:8. Strong staining is evident in surface regions in very similar pattern to that shown for rabbit Ig. $\times 250$.

injected antigen was demonstrated in elution experiments (Table IV). At 2, 4, and 6 wk after induction, precipitable anti-BSA counts represented 90% of the extracted radioactivity. Preliminary results using Sephadex G-100 columns indicate that most of the extracted antigen appears to have the molecular size of the native protein.³

In the elution studies, it was consistently impossible to extract antigen with acid buffers or 3 M $MgCl_2$, the latter being known to release 80% of the chondromucoprotein in bovine nasal cartilage over a 24 hr period (4). Since it was also shown that the major fraction of antigen was present in ligamentous structures and only to a lesser extent in hyaline cartilage, it would appear that the

³ Jasin, H. E., T. D. Cooke, and M. Ziff. Unpublished observations.



antigen may be bound to collagen and not to interfibrillar matrix. The radioactivity in juxta-articular bone appears to be due to residual attachment of ligaments and articular cartilage.

The immunofluorescence studies have indicated the presence of Ig on the articular surface of collagenous tissue. The distribution of the Ig coincided with that demonstrated for antigen, both by radioautography and by immunofluorescent staining, suggesting that this Ig was specific antibody complexed with the antigen. This finding was supported by the fact that antigen was selectively bound when incubated *in vitro* with intact menisci from arthritic animals immunized to that antigen. This experiment also indicated that the antibody portion of the deposited immune complexes was able to interact readily with the fluid phase of the joint cavity. Furthermore, demonstration of a complement component, C3, in an identical location with Ig and antigen suggested that it was fixed to immune complexes.

The data to this point indicate that the inducing antigen persists, complexed with antibody and complement in a sequestered state on the articular surface of the collagenous tissue of the joint. In a previous paper,² we showed that the synovium maintained a prolonged active synthesis of Ig and that the antibody synthesized was largely directed against the specific inducing antigen. Since it is likely that a continuing immune response requires the continued presence of antigen (6), the present observations of chronic specific antibody synthesis would appear to be the consequence of persisting antigen in the joint. The persistence of antigen observed may be due to its association to excess antibody in collagenous tissue, a state in which it may be protected from attack by the proteolytic enzymes of synovial fluid, since the Fab fragments of the antibody are known to be resistant to such degradation (7, 8).

The capacity of antigen persisting in the joints of normal animals to produce a local synovitis when they are subsequently immunized has been demonstrated by Webb et al. (9). These workers observed that when EA was injected into the joints of normal rabbits, and these animals were subsequently immunized with this antigen at regular intervals up to 4 wk later, synovitis developed in all the antigen-injected joints but not in saline-injected controls. Very similar evidence of the capabilities of persisting antigen has been demonstrated in

FIG. 10. Meniscus from knee of rabbit injected IA 2 wk previously with saline solution. Stained with fluorescein-conjugated goat anti-rabbit Ig diluted 1:2. Note the lack of specific surface staining of the cartilage. Nonspecific fluorescence of chondrocytes is evident at this concentration of fluoresceinated antiserum. $\times 250$.

FIG. 11. Photomicrograph of articular cartilage from a joint with acute gout induced 30 hr previously by IA injection of monosodium urate crystals. Stained with fluorescein-conjugated anti-rabbit Ig diluted 1:2. No surface staining is evident. Nonspecific chondrocyte fluorescence is seen. $\times 250$.

FIG. 12. Photomicrograph of meniscus from a joint injected 2 wk previously with saline solution and stained with guinea pig anti-rabbit C3, diluted 1:2, and fluorescein-conjugated rabbit anti-guinea pig Ig diluted 1:8. Note the absence of staining. $\times 250$.

experimental corneal hypersensitivity (10). In these experiments even very small amounts of antigen could evoke an inflammatory reaction, once antibody had made its appearance in subsequently immunized animals.

The presence of complement-binding immune complexes on the articular surface of collagenous tissue may be expected to be associated with the occurrence of joint inflammation since such complexes can activate complement and give rise to chemotactic factors for polymorphonuclear cells (11, 12). Activation of complement and the production of chemotactic factors in the synovial fluid has been observed in rheumatoid arthritis (13, 14).

The present findings also recall studies (15, 16) dealing with the fate of antigens in germinal centers of lymphoid organs of immunized animals. These have shown that antigen and antibody, presumably in the form of immune complexes, are observed in a mesh-like pattern in the germinal centers. In this form, antigens persist for long periods of time in intercellular spaces in close contact with dendritic, nonphagocytic reticular cells (17). It has been pointed out (18) that the dendritic processes are in intimate contact with the reticulin fibers in the lymph nodes, which are composed of collagen fibrils and proteoglycan. It is possible, therefore, that, as postulated in cartilage and the other collagenous tissues, the antigen-antibody complexes in germinal centers may be associated with collagen fibers. Haurowitz (19) has in fact suggested that an association of persisting antigen to proteins with a slow turnover rate, of which collagen is an example, may provide an explanation for its slow elimination. A similar mechanism may play a role in the localization of immune complexes in the basal membranes of skin (20) and renal glomeruli (21-23) in human pathology. In these sites, antigen-antibody complexes are trapped in acellular structures containing polysaccharide and collagen-like material. The binding of complexes in these sites, where they would be protected from cellular or enzymatic attack, would permit their persistence for long periods of time.

The observation in the present experiments, that persisting antigen, complexed with antibody, is bound to the surface of collagenous tissue in the joint has raised the question of a similar localization of immune complexes in rheumatoid arthritis. It is of interest that preliminary studies⁴ have demonstrated immunofluorescent staining of IgG, IgM, and C3 in the articular cartilage of seven of eight patients with rheumatoid arthritis. Such complexes could play a significant role in this disease by providing a reservoir of antigen, either exogenous or endogenous, for the maintenance of joint inflammation for prolonged periods of time. They might also play a role in the erosion of cartilage and tendons through the liberation of chemotactic factors in the extracellular space of these tissues, thus attracting polymorphonuclear cells and hastening their degeneration.

⁴ Cooke, T. D., E. H. Hurd, H. Tateishi, H. E. Jasin, and M. Ziff. Unpublished observations.

SUMMARY

In an experimental arthritis induced by injection of bovine serum albumin or egg albumin into the joints of previously immunized animals, it has been demonstrated that the major portion of the radioactively labeled antigens injected was localized to avascular collagenous tissues in the joint, i.e., articular cartilage, menisci, and intra-articular ligaments. The antigens were partially eluted from the tissues with 5 M guanidine solution, but not with acid buffers or by 3 M magnesium chloride. The radioactive material eluted with guanidine was at least 80% precipitable by specific antisera.

The radioactively labeled-inducing antigen was identified on the surface of articular collagenous tissues from arthritic joints by radioautography and immunofluorescence. Rabbit immunoglobulin and C3 were demonstrated in the same sites by immunofluorescence. The presence of specific antibody in collagenous tissues was demonstrated by the selective *in vitro* binding of ¹²⁵I-labeled-inducing antigen to menisci from arthritic joints of immunized animals.

The evidence obtained indicates that in this model of chronic arthritis, the inducing antigen persists for long periods of time in the form of immune complexes in the surface layers of the intra-articular collagenous tissue. The antigen retained in this form may be responsible for the chronicity of the synovitis by serving as a direct stimulus for the maintenance of prolonged antibody synthesis in the synovium and by providing a source of complement-fixing antigen-antibody complexes for the mediation of joint inflammation.

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